high $_eK^+$ (1–12 min after treatment with EGTA) had an additive effect on cellular O_2 consumption, reducing $_iO_2$ to 60% of air saturation. The respiratory response was accompanied by Na^+ influx, a decrease in cytosolic and mitochondrial Ca^{2+} and partial depolarization of plasma membrane, while the mitochondrial membrane potential (MMP) and cellular ATP remained unchanged. The effect of EGTA was down-regulated by the depletion of Ca^{2+} stores and dissipation of proton gradient across the mitochondrial membrane, up-regulated by mitochondria uncoupling and was independent on MMP. The respiratory effect was largely reduced by the inhibition of mitochondrial Na^+/Ca^{2+} exchanger ($_mNCX$) and Na^+/H^+ exchangers. We suggest that such respiratory response is driven by a non-selective Na^+ influx, activation of $_mNCX$ and increased mitochondrial Na^+/H^+ exchange. This leads to the acidification of matrix, loss of $_mCa^{2+}$ and acceleration of mitochondrial proton pumps to restore proton gradient.

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S7.10 Bioenergetics and mitochondrial transport in hippocampal neurons

Akos A. Gerencser[a,b], David G. Nicholls[a]
[a]*Buck Institute for Age Research, Novato, CA, USA*
[b]*Semmelweis University, Department of Medical Biochemistry, Budapest, Hungary*
E-mail: agerencser@buckinstitute.org

Impaired transport of mitochondria in neurons and bioenergetic deficit are increasingly recognized to be of pathological importance in neurodegenerative diseases. To study the relationship between transport and bioenergetics we have developed a novel image processing technique to quantify organelle velocity in cultured cells. This combines measurement of motion and bioenergetic parameters while minimizing photodynamic oxidative artifacts evoked by fluorescence excitation. To describe populations of mitochondria in resting cultured hippocampal neurons in addition to motion analysis, measurements of mitochondrial thiol redox status by mitochondrially-targeted redox-sensitive GFP and mitochondrial membrane potential by TMRM were performed. Mitochondria with more oxidized thiol redox status had lower membrane potentials and were smaller in size. These mitochondria were more motile than the average, however mitochondrial motility was only slightly dependent on the observed bioenergetic parameters, and correlated the best to their size. Mean velocities of mitochondria were unaltered by glycolytic inhibition and decreased by inhibition of oxidative phosphorylation. To stop motion cessation of both ATP sources was required. Depolarization of mitochondria when the ATP-synthase was inhibited did not further decrease the mean velocity and affect the directionality of the motion. It is concluded that mitochondrial motors respond to the global ATP level, which is mainly determined by the oxidative phosphorylation. The mitochondrial membrane potential does not regulate mitochondrial transport in hippocampal neurons.

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S7.11 Metabolic control analysis of bioenergetic function in synaptosomes

Jayne Telford[a,b], Michael J. Rowan[b,c], Keith F. Tipton[a], Martha Motherway Gildea[a], Gavin P. Davey[a,b]
[a]*School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Ireland*
[b]*Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland*
[c]*Department of Pharmacology and Therapeutics, Trinity College Dublin, Dublin 2, Ireland*
E-mail: telforj@tcd.ie

The aim of this study was to use metabolic control analysis (MCA) to examine the spread of control amongst the electron transport chain (ETC) complexes over the process of mitochondrial oxidative phosphorylation in rat brain synaptosomes. Oxygen consumption and ETC activities were titrated with appropriate inhibitors to determine the flux control coefficients and the energy threshold levels. The flux control coefficients for complex I, complex II/III and complex IV were found to be 0.30, 0.20 and 0.19, respectively and the energy thresholds for complex I, complex II/III and complex IV were determined to be ~15%, ~35 and ~30%, respectively. These results indicate that complex I exerts a high level of control over synaptosomal bioenergetics, suggesting that complex I deficiencies in neurodegenerative disorders, such as PD, may compromise mitochondrial oxygen consumption in the nerve terminal, possibly leading to neuronal dysfunction. In addition, the effect of coenzyme Q on the flux control coefficient and energy threshold effect of complex I was examined. No statistically significant difference in flux control coefficients and energy thresholds for complex I was found, however, during titration of complex I activity with rotenone the presence of coenzyme Q decreased the rate of inhibition of oxygen respiration. These results suggest that complex I in the nerve terminal possess sensitive control over mitochondrial respiration rates and may be a therapeutic target for neurodegenerative conditions in which complex I activities are decreased.

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(S8) Mitochondria and cell physiology symposium lecture abstracts

S8/1 Iodothyronines and mitochondria

Fernando Goglia
Università degli studi del Sannio-Dip.to di Scienze Biologiche-Benevento, Italy
E-mail: goglia@unisannio.it

Studies of the effects of iodothyronines on mitochondria have been focused on T3, but recently other iodothyronines such 3,5-diiodothyronine (T2), have been identified as possible peripheral mediators of the effect of thyroid hormones on cell respiration. In this context, we have shown that T2 powerfully reduce adiposity in high-fat-fed rats by increasing the burning of fats. Now we report that T2 in a short term is able i) to affect mitochondrial fatty acid oxidation rate in skeletal muscle ii) to activate the AMPK-(ACC)-malonyl CoA signalling pathway iii) to affect mitochondrial thermogenesis. The administration of T2 to hypothyroid rats induced an increase in mitochondrial oxidation when palmitoyl-CoA (+104% vs. hypo), palmitoyl-carnitine (+80% vs. hypo) and succinate (+30% vs. hypo) were used as substrates. These results suggest that T2 stimulates mitochondrial fatty acid oxidation by activating more metabolic pathways: -import of fatty acid into the mitochondria–beta oxidation cycle–$FADH_2$ linked respiratory pathways. Indeed, T2 is able to activate the AMPK signalling pathway known to direct lipid partition towards oxidation and to induce the activation of mitochondrial fatty acid import. T2 also enhanced skeletal muscle mitochondrial thermogenesis by activating pathways involved in the dissipation of proton motive force not associated to ATP synthesis ("proton leak"), the effect being

dependent on the presence of free fatty acid. Conclusions: by activating processes enhancing fatty acid oxidation T2 could protect skeletal muscle against lipotoxicity.

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S8/2 In situ oxidative phosphorylation, oxidative stress, and mitochondrial morphology of INS-1E and HEP-G2 cells

Petr Ježek, Lydie Hlavatá-Plecitá
Department 75, Institute of Physiology, Academy of Sciences, Prague, Czech Republic
E-mail: jezek@biomed.cas.cz

We have used 4Pi microscopy 3D imaging (~250 nm lateral and ~100 nm axial resolution) to demonstrate that cells relying on intensive oxidative phosphorylation contain in fact a single mitochondrion, a slightly branched dense mitochondrial reticulum filling the substantial cell volume. Unlike conventional confocal microscopy, resolving apparently tubules of ~800 nm diameter, we clearly show an average tubule diameter of 262 nm in insulinoma INS-1E cells and 284 m in hepatocellular carcinoma HEP-G2 cells cultivated at 5 mM glucose. Moreover, mitochondrial reticulum shapes resulting from fission induction by decreasing OXPHOS cannot originate from a sole fission but must originate also from concomitant fusion, since e.g. uncoupling led to rings, obviously arisen from fusion of two ends of short segments, while uncoupling at an inhibited respiratory chain led to rings with closed outlets, i.e. to vessel type objects, where fusion must be even more prominent. HEP-G2 cells cultivated at 25 mM glucose exhibited thicker tubules but also lower matrix-released superoxide production, the un-dismuted surplus (J_m) confocally indicated by MitoSOX. Rotenone caused a 5-fold J_m increase, completely attenuated by uncoupling and by MitoQ. A hydrophobic amiloride that acts on the ND5 subunit and inhibits Complex I H^+ pumping enhanced J_m and even countered the attenuating effect of FCCP, but not that of MitoQ.

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S8/3 Mitochondrial respiratory physiology: Convergent electron transport system and flux control of oxidative phosphorylation in intact and permeabilized cells

Erich Gnaiger
Medical University of Innsbruck, Department of General and Transplant Surgery, D. Swarovski Research Laboratory, 6020 Innsbruck, Austria
E-mail: erich.gnaiger@i-med.ac.at

Oxidative phosphorylation (OXPHOS) is a key element of bioenergetics, extensively studied to resolve mechanisms of energy transduction and respiratory control in the electron transport system (ETS). Electrons flow to oxygen from Complex I or II with three or two coupling sites. The functional design of the ETS was studied in permeabilized NIH3T3 fibroblasts by high-resolution respirometry with multiple substrate-uncoupler-inhibitor titration protocols. Compared to *ETS* capacity in intact cells, conventional State 3 respiration in permeabilized cells was only 0.38±0.06 with ADP and glutamate +malate. *ETS* capacities were identical in intact and permeabilized uncoupled cells, however, with convergent electron flow to the Q-junction from glutamate+malate+succinate through Complexes I and II (CI+II e-input). Coupled *OXPHOS* flux was 0.50±0.09 of *ETS* capacity, reflecting control of the phosphorylation system over *OXPHOS*. Convergent CI+II e-input provides the relevant basis for quantifying enzymatic thresholds and excess capacities of individual steps of *OXPHOS*, and for evaluation of mitochondrial defects. Convergent CI+II e-input corresponds to operation of the tricarboxylic acid cycle and mitochondrial substrate supply *in vivo* and yields novel insights into the physiological diversity of mitochondria from various tissues. Multiple substrate-uncoupler-inhibitor titration protocols extend the diagnostic potential of mitochondrial physiology in health and disease.

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(S8) Mitochondria and cell physiology symposium abstracts (poster and raised abstracts)

S8.4 Mitochondrial superoxide generation is diminished during glucose-stimulated insulin secretion in INS-1E cells

Tomáš Špaček, Lydie Plecitá-Hlavatá, Petr Ježek
Department 75, Institute of Physiology, Academy of Sciences, Prague, Czech Republic
E-mail: spacek@biomed.cas.cz

One of the unique features of β-cells lies in their relatively low expression of antioxidant enzymes. It makes them liable to oxidative damage — one of etiologies for type 2 diabetes development. Using matrix-localized MitoSOX, we have monitored excessive superoxide production released to the mitochondrial matrix (J_m) in insulinoma INS-1E cells before and after glucose addition, i.e. under glucose-stimulated insulin secretion (GSIS) conditions. Independently of the original glucose level (cells cultivated at 11 mM or 3 mM glucose) J_m substantially decreased upon glucose addition. % decrease in J_m was linearly dependent on the incremental glucose in mM. J_m was also suppressed by an uncoupler or a fatty acid, showing attenuating effects of mild uncoupling. Since previously we have demonstrated increasing ATP synthesis (OXPHOS) with increasing glucose added to glucose-depleted INS-1E cells, saturating above 12 to 15 mM glucose, our data indicate that increasing OXPHOS and concomitantly increasing H^+ backflow across the F_O part of ATP synthase attenuates mitochondrial superoxide production including that on Complex I. We conclude that GSIS does not induce oxidative stress in mitochondrial matrix *in situ* but actually attenuates superoxide production established at mild starvation. Supported by grants NR/9183 - 3; IAA500110701.

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S8.5 Regulation of oxidative phosphorylation in response to graded uncoupling towards the limit of electron transport capacity

Patrick Subarsky[a,b], Erich Gnaiger[a]
[a]*Department of General and Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck Medical University, Austria*
[b]*Department of Medical Biophysics, University of Toronto, Canada*
E-mail: p.subarsky@utoronto.ca

Mitochondrial oxygen consumption is divided between the support of ADP phosphorylation and *LEAK* (including proton leak through the inner membrane and proton slip in the respiratory complexes). The aim of our study was to determine the distribution of oxygen consumption between the two processes in intact cells (32D, myeloblast-like). Electron transport capacity (E) was defined as the maximum respiration under conditions of optimal FCCP concentration (76.2±12.9 pmol O_2 s^{-1} per 10^6 cells). Cell respiration (R) under